Title

PREPARATION OF A LIPID BLEND AND A PHOSPHOLIPID SUSPENSION CONTAINING THE LIPID BLEND

Cross-Reference to Related Applications

This application is a continuation of co-pending U.S. application Serial No. 09/229,258, filed January 13, 1999, which is a non-provisional filing of U.S. provisional application Serial No. 60/071,332, filed January 14, 1998. Right to priority of these earlier applications, which are incorporated herein by reference in their entirety, for all purposes, is hereby claimed.

Field of the Invention

The present invention relates generally to processes for the preparation of a lipid blend and a uniform filterable phospholipid suspension containing the lipid blend, such suspension being useful as an ultrasound contrast agent.

Background of the Invention

Manufacturing of a phospholipid contrast agent can be divided into the following steps: (1) preparation of lipid blend; (2) compounding the bulk solution, which involves the hydration and dispersion of the lipid blend in an essentially aqueous medium to produce a lipid suspension; (3) filtration of the bulk solution through a sterilizing filter(s) to render the suspension free of microbial contaminants; (4) dispensing the sterile suspension into individual vials in a controlled aseptic area; (5) loading the dispensed vials into a lyophilizer chamber to replace the vial headspace gas with perfluoropropane gas (PFP); (6) transferring the sealed vials after gas exchange to an autoclave for terminal sterilization. There are three major obstacles in this process: (1) uniformity of the lipid blend; (2) hydration of the lipid blend; (3) uniformity and

particle size of the suspension; and, (4) sterile filtration of the suspension through a sterilizing filter(s).

Phospholipid blends are typically produced by dissolving or suspending the required lipids in an appropriate aqueous or non-aqueous solvent system, and then reducing the volume either by lyophilization or distillation. Ideally, this process produces blended solids with high content uniformity and purity. However, while working well on a small, laboratory scale, this simple approach is frequently problematic upon scale-up to production-size quantities. Difficulties include: (1) maintaining content uniformity during the solvent removal step (due to differential solubilities); (2) maintaining purity (frequently a problem when water is used due to hydrolytic side-reactions); (3) enhancing purity; (4) minimizing solvent volume; and (5) recovery of the final solids (e.g., it is not practical to scrape solids out of a large reactor).

After manufacture of a lipid blend, final compounding typically involves introduction of the blend into an aqueous medium. Since phospholipids are hydrophobic and are not readily soluble in water, adding phospholipids or a lipid blend directly into an aqueous solution causes the lipid powder to aggregate forming clumps that are very difficult to disperse. Thus, the hydration process cannot be controlled within a reasonable process time. Direct hydration of phospholipids or a lipid blend in an aqueous medium produces a cloudy suspension with particles ranging from 0.6 μm to to 100 μm. Due to relatively large particle size distribution, the suspension cannot be filtered at ambient temperature when the suspension solution temperature is below the gel-to-liquid crystal phase transition temperatures of lipids. The lipids would accumulate in the filters causing a restriction in the flow rate, and in most cases, the filters would be completely blocked shortly after. Further reduction in the suspension particle size cannot be achieved through a conventional batching process, even after extended mixing (e.g., 6 hours) at elevated

temperatures (e.g., 40°C to 80°C) with a commonly used marine propeller.

Although filtration at elevated temperatures, i.e., at above the phase transition temperatures of lipids, is possible, a significant amount of larger lipid particles would still be excluded when a normal filtering pressure is used. In turn, concentrations of the sterile filtrate would have variable lipid content from batch to batch depending on how the lipids are initially hydrated which is in turn determined by the physical characteristics, e.g., morphology, of the starting materials.

The process of directly hydrating the lipids or lipid blend to produce a uniform suspension and filtration of the suspension through a sterilization filter(s) can be difficult and costly to be scaled-up to any reasonable commercial scale, e.g., >20L.

Thus, the presently claimed processes for manufacture of a lipid blend and the subsequent phospholipid suspension are aimed at solving the above issues by providing a practical process that can be easily scaled and adopted to various manufacturing facilities without extensive modification or customization of existing equipment.

Summary of the Invention

Accordingly, one object of the present invention is to provide a novel process for preparing a lipid blend.

Another object of the present invention is to provide a novel process for preparing a phospholipid suspension from the lipid blend.

These and other objects, which will become apparent during the following detailed description, have been achieved by the inventors' discovery that dissolving a lipid blend in a suitable non-aqueous solvent prior to introduction of an aqueous solution allows for production of a phospholipid suspension.

Detailed Description of the Invention

[1] Thus, in a first embodiment, the present invention provides a novel process for preparing a phospholipid suspension, comprising:

(1) contacting a lipid blend with a non-aqueous solvent, whereby the lipid blend substantially dissolves in the non-aqueous solvent; and,

(2) contacting the solution from step (1) with an aqueous solution to form a lipid suspension.

[2] In a preferred embodiment, the non-aqueous solvent is selected from propylene glycol, ethylene glycol, and polyethylene glycol 300.

[3] In a more preferred embodiment, the non-aqueous solvent is propylene glycol.

[4] In another preferred embodiment, the lipid blend, comprises:

(a) 1,2-dipalmitoyl-*sn*-glycero-3-phosphatidylcholine;

(b) 1,2-dipalmitoyl-*sn*-glycero-3-phosphotidic, mono sodium salt; and,

(c) *N*-(methoxypolyethylene glycol 5000 carbamoyl)-1,2-dipalmitoyl-*sn*-glycero-3-phosphatidylethanolamine, mono sodium salt.

[5] In another preferred embodiment, in step (1), the non-aqueous solvent is heated to a temperature of about 30 to 70°C prior to contacting with the lipid blend.

[6] In another more preferred embodiment, the non-aqueous solvent is heated to a temperature of about 50 to 55°C prior to contacting with the lipid blend.

[7] In another preferred embodiment, the ratio of lipid blend to non-aqueous solvent is from about 5 mg of lipid blend per mL of non-aqueous solvent to about 15 mg/mL.

[8] In another more preferred embodiment, the ratio of lipid blend to non-aqueous solvent is about 10 mg/mL.

[9] In another preferred embodiment, in step (2), the aqueous solution is selected from water, saline, a saline/glycerin mixture, and a saline/glycerin/non-aqueous solvent mixture.

[10] In another more preferred embodiment, the aqueous solution is a saline and glycerin mixture.

[11] In another more preferred embodiment, the aqueous solution is a saline, glycerin, and propylene glycol mixture.

[12] In another more preferred embodiment, 6.8 mg/mL of sodium chloride are present, 0.1 mL/mL of glycerin are present, 0.1 mL/mL of propylene glycol are present, and about 0.75 to 1.0 mg/mL of the lipid blend are present.

[13] In an even more preferred embodiment, 0.75 mg/mL of lipid blend are present.

[14] In another more preferred embodiment, 1.0 mg/mL of lipid blend are present.

[15] In another preferred embodiment, in step (2), the aqueous solution is heated to a temperature of about 45 to 60°C prior to contacting with the solution from step (1).

[16] In another more preferred embodiment, the aqueous solution is heated to a temperature of about 50 to 55°C prior to contacting with the solution from step (1).

[17] In another preferred embodiment, the process further comprises:

(3) heating the lipid suspension from step (2) to a temperature about equal to or above the highest gel to

liquid crystalline phase transition temperature of the lipids present in the suspension.

[18] In another more preferred embodiment, in step (3), the lipid suspension is heated to a temperature of at least about 67°C.

[19] In another more preferred embodiment, the process further comprises:

(4) filtering the lipid suspension through a sterilizing filter.

[20] In another even more preferred embodiment, in step (4), the filtration is performed using two sterilizing filter cartridges.

[21] In a further preferred embodiment, in step (4), the sterilizing filter cartridges are at a temperature of from about 70 to 80°C.

[22] In another further preferred embodiment, in step (4), 0.2μm hydrophilic filters are used.

[23] In another even more preferred embodiment, the process further comprises:

(5) dispensing the filtered solution from step (4) into a vial.

[24] In another further preferred embodiment, the process further comprises:

(6) exchanging the headspace gas of the vial from step (5) with a perfluorocarbon gas.

[25] In another even further preferred embodiment, the perfluorocarbon gas is perfluoropropane.

[26] In another even further preferred embodiment, exchange of headspace gas is performed using a lyophilizing chamber.

[27] In another even further preferred embodiment, the process further comprises:

(7) sterilizing the vial from step (6).

[28] In a still further preferred embodiment, in step (7), the vial is sterilized at about 126-130°C for 1 to 10 minutes.

[29] In a second embodiment, the present invention provides a novel process for preparing a lipid blend, comprising:

(a) contacting at least two lipids with a first non-aqueous solvent;

(b) concentrating the solution to a thick gel;

(c) contacting the thick gel with a second non-aqueous solvent; and,

(d) collecting the resulting solids.

[30] In a preferred embodiment, in step (a), the lipids are:

(i) 1,2-dipalmitoyl-*sn*-glycero-3-phosphatidylcholine;

(ii)1,2-dipalmitoyl-*sn*-glycero-3-phosphotidic, mono sodium salt; and,

(iii) *N*-(methoxypolyethylene glycol 5000 carbamoyl)-1,2-dipalmitoyl-*sn*-glycero-3-phosphatidylethanolamine, mono sodium salt.

[31] In another preferred embodiment, in step (a), the first non-aqueous solvent is a mixture of methanol and toluene.

[32] In another preferred embodiment, in step (c), the second non-aqueous solvent is a methyl *t*-butyl ether.

[33] In another preferred embodiment, in step (a), the solution is warmed to a temperature sufficient to complete dissolution of the lipids into the solvent.

[34] In another more preferred embodiment, in step (a), the solution is warmed to about 25 to 75°C.

[35] In another preferred embodiment, in step (d), the solids collected are washed with methyl *t*-butyl ether and dried *in vacuo*.

[36] In a third embodiment, the present invention provides a novel phospholipid suspension, comprising:

(a) a lipid blend in an amount of about 0.75 - 1.0 mg/mL of suspension;

(b) sodium chloride in an amount of about 6.8 mg/mL of suspension;

(c) glycerin in an amount of about 0.1 mL/mL of suspension;

(d) propylene glycol in an amount of about 0.1 mL/mL of suspension; and

(e) water;

wherein the suspension is prepared by the process, comprising:

(1) contacting a lipid blend with a non-aqueous solvent, whereby the lipid blend substantially dissolves in the non-aqueous solvent;

(2) contacting the solution from step (1) with an aqueous solution to form a lipid suspension;

(3) heating the lipid suspension from step (2) to a temperature about equal to or above the highest gel to liquid crystalline phase transition temperature of the lipids present in the suspension; and,

(4) filtering the lipid suspension through a sterilizing filter.

[37] In another preferred embodiment, the lipid blend, comprises:

(a) 1,2-dipalmitoyl-*sn*-glycero-3-phosphatidylcholine;

(b) 1,2-dipalmitoyl-*sn*-glycero-3-phosphotidic, mono sodium salt; and,

(c) *N*-(methoxypolyethylene glycol 5000 carbamoyl)-1,2-dipalmitoyl-*sn*-glycero-3-phosphatidylethanolamine, mono sodium salt.

[38] In another more preferred embodiment, the non-aqueous solvent is heated to a temperature of about 50 to 55°C prior to contacting with the lipid blend.

[39] In another more preferred embodiment, the ratio of lipid blend to non-aqueous solvent is about 10 mg/mL.

[40] In another more preferred embodiment, the aqueous solution is a saline, glycerin, and propylene glycol mixture.

[41] In an ever more preferred embodiment, 0.75 mg/mL of lipid blend are present.

[42] In another more preferred embodiment, the aqueous solution is heated to a temperature of about 50 to 55°C prior to contacting with the solution from step (1).

[43] In another more preferred embodiment, in step (3), the lipid suspension is heated to a temperature of at least about 67°C.

[44] In another further preferred embodiment, in step (4), two 0.2μm hydrophilic filters are used.

Formulation

The present invention is contemplated to be practiced on at least a multigram scale, kilogram scale, multikilogram scale, or industrial scale. Multigram scale, as used herein, is preferably the scale wherein at least one starting material is present in 10 grams or more, more preferably at least 50 grams or more, even more preferably at least 100 grams or more. Multikilogram scale, as used herein, is intended to mean the scale wherein more than one

kilogram of at least one starting material is used. Industrial scale as used herein is intended to mean a scale which is other than a laboratory scale and which is sufficient to supply product sufficient for either clinical tests or distribution to consumers.

Lipid blend or phospholipid blend, as used herein, is intended to represent two or more lipids which have been blended. The lipid blend is generally in a powder form. Preferably, at least one of the lipids is a phospholipid. Preferably, the lipid blend contains 1,2-dipalmitoyl-*sn*-glycero-3-phosphatidylcholine (DPPC), 1,2-dipalmitoyl-*sn*-glycero-3-phosphotidic, mono sodium salt (DPPA), and *N*-(methoxypolyethylene glycol 5000 carbamoyl)-1,2-dipalmitoyl-*sn*- glycero-3-phosphatidylethanolamine, monosodium salt (MPEG5000-DPPE). The amount of each lipid present in the blend will depend on the desired end product. Preferred ratios of each lipid are described in the Examples section. A wide variety of other lipids, like those described in Unger et al, U.S. Patent No. 5,469,854, the contents of which are hereby incorporated by reference, may be used in the present process.

Phospholipid, as used herein, is a fatty substance containing an oily (hydrophobic) hydrocarbon chain(s) with a polar (hydrophilic) phosphoric head group. Phospholipids are amphiphilic. They spontaneously form boundaries and closed vesicles in aqueous media. Phospholipids constitute about 50% of the mass of animal cell plasma membrane.

Preparation of the lipid blend

The lipid blend may be prepared via an aqueous suspension-lyophilization process or an organic solvent dissolution-precipitation process using organic solvents. In the aqueous suspension-lyophilization process, the desired lipids are suspended in water at an elevated temperature and then concentrated by lyophilization. Preferably a dissolution procedure is used.

Step (a):

The organic solvent dissolution-precipitation procedure involves contacting the desired lipids (e.g., DPPA, DPPC, and MPEG5000 DPPE) with a first non-aqueous solvent system. This system is typically a combination of solvents, for example $CHCl_3$/MeOH, CH_2Cl_2/MeOH, and toluene/MeOH. Preferably, the first non-aqueous solvent is a mixture of toluene and methanol. It may be desirable to warm the lipid solution to a temperature sufficient to achieve complete dissolution. Such a temperature is preferably about 25 to 75°C, more preferably about 35 to 65°C.

After dissolution, it may be desired to remove undissolved foreign matter by hot-filtration or cooling to room temperature and then filtering. Known methods of filtration may be used (e.g., gravity filtration, vacuum filtration, or pressure filtration).

Step (b):

The solution is then concentrated to a thick gel/semisolid. Concentration is preferably done by vacuum distillation. Other methods of concentrating the solution, such as rotary evaporation, may also be used. The temperature of this step is preferably about 20 to 60°C, more preferably 30 to 50°C.

Step (c):

The thick gel/semisolid is then dispersed in a second non-aqueous solvent. The mixture is slurried, preferably near ambient temperature (e.g., 15-30°C). Useful second non-aqueous solvents are those that cause the lipids to precipitate from the filtered solution. The second non-aqueous solvent is preferably methyl *t*-butyl ether (MTBE). Other ethers and alcohols may be used.

Step (d):

The solids produced upon addition of the second non-aqueous solvent are then collected. Preferably the collected solids are washed with another portion of the

second non-aqueous solvent (e.g., MTBE). Collection may be performed via vacuum filtration or centrifugation, preferably at ambient temperature. After collection, it is preferred that the solids are dried *in vacuo* at a temperature of about 20-60°C.

For the following reasons, the organic solvent dissolution-precipitation process is preferred over the aqueous suspension/lyophilization process:

(1) Because the lipids are quite soluble in toluene/methanol, solvent volumes are significantly reduced (relative to the aqueous procedure).

(2) Because of this increased solubility, the process temperature is also lower relative to the aqueous procedure, thereby avoiding the hydrolytic instability of fatty acid esters.

(3) When cooled back to room temperature, the toluene/methanol solution of lipids remains homogeneous, allowing a room temperature filtration to remove solid foreign matter.

(4) The MTBE precipitation allows quick and easy isolation of Lipid Blend solids. With the aqueous process, a time-consuming lyophilization process is used to isolate material.

(5) The MTBE precipitation also allows for the removal of any MTBE-soluble impurities, which go into the filtrate waste-stream. This potential for impurity removal is not realized when a solution is directly concentrated or lyophilized to a solid.

(6) The present process affords uniform solids.

<u>Preparation of the lipid suspension</u>

<u>Step (1):</u>

In step one, a lipid blend is contacted with a non-aqueous solvent, whereby the lipid blend substantially dissolves in the non-aqueous solvent. Alternatively, the individual lipids may be contacted with the non-aqueous solvent sequentially in the order: DPPC, DPPA, and

MPEG5000-DPPE; DPPC, MPEG5000-DPPE, and DPPA; MPEG5000-DPPE, DPPA, and DPPC; or MPEG5000-DPPE, DPPC, and DPPA. The DPPA, being the least soluble and least abundant of the lipids is not added first. Adding one of the other lipids prior to or concurrently with adding the DPPA facilitates dissolution of the DPPA. In another alternative, the individual lipids can be combined in their solid forms and the combination of the solids contacted with the non-aqueous solvent.

Substantial dissolution is generally indicated when the mixture of lipid blend and non-aqueous solven becomes clear. As noted previously, phospholipids are generally not water soluble. Thus, direct introduction of a blend of phospholipid blend into an aqueous environment causes the lipid blend to aggregate forming clumps that are very difficult to disperse. The present invention overcomes this limitation by dissolving the lipid blend in a non-aqueous solvent prior to introduction of the aqueous solution. This allows one to evenly disperse the lipid blend into a liquid. The liquid dispersion can then be introduced into a desired aqueous environment.

Non-aqueous is intended to mean a solvent or mixture of solvents wherein the amount of water present is sufficiently low as to not impede dissolution of the lipid blend. The amount of non-aqueous solvent required will depend on the solubility of the lipid blend and also the final desired concentration of each component. As one of ordinary skill would appreciate, the level of water present in the non-aqueous solvent, which may be tolerated will vary based on the water solubilities of the individual lipids in the lipid blend. The more water soluble the individual phospholipids, the more water which may be present in step (1). Preferably, propylene glycol is used as the non-aqueous solvent. However, other members of the polyol family, such as ethylene glycol, and polyethylene glycol 300 may be used.

Mechanically mixing the lipid blend and non-aqueous solvent may be necessary to achieve complete dissolution. One of ordinary skill in the art will recognize that a

variety of ways of mixing are available. It is preferred that a high shear homogenizer is used.

One of ordinary skill in the art would recognize that raising the temperature of the solvent should aid in dissolution of the lipid blend. The temperature at which step (1) may be performed can range from ambient to the boiling point of the chosen solvent. Preferably the temperature is from about 30 to about 70°C, more preferably about 45 to about 60°C, and even more preferably about 50, 51, 52, 53, 54, or 55°C. When ethylene glycol or polyethylene glycol 300 is used, it is preferred that the temperature be from about 50 to about 60°C and more preferably about 55°C. Maintaining the solution at an elevated temperature should reduce solution viscosity and ease formulation preparation.

A preferred procedure for dissolving the lipid blend is as follows: (a) Add propylene glycol to an appropriate weighing container. (b) Warm the propylene glycol to about 40-80°C in a heating bath. (c) Weigh the lipid blend into a separate container. (d) When the propylene glycol has reached the desired temperature range, transfer the solution into the container containing the lipid blend. (e) Place the container back into the heating bath until the solution is clear. (f) Mechanically mix the Lipid Blend/Propylene Glycol solution to further assure complete dissolution and uniform dispersion of the lipid blend.

The ratio of lipid blend to non-aqueous solvent will, of course, be limited by the solubility of the lipid blend. This ratio will also be influenced by the desired amount of lipid blend in the final formulation. Preferably, the ratio is from about 1 mg of lipid blend per mL of solvent (mg/mL) to about 100 mg/mL. More preferably, the lipid blend is present in about 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15 mg/mL. Even more preferably, the lipid blend is present in about 10 mg/mL.

Step (2):

The second step involves contacting the solution from step (1) with an aqueous solution to form a lipid suspension. The aqueous solution can be water, saline, a saline/glycerin mixture or a saline/glycerin/non-aqueous solvent mixture. Non-aqueous solvent is as defined previously, preferably propylene glycol. Suspension, as used herein, is intended to indicate a dispersion in which insoluble particles are dispersed in a liquid medium.

Once complete dissolution of the lipid blend has been achieved (step (1)), the resulting solution can then be introduced to an aqueous solution. The aqueous solution may contain one or more components selected from sodium chloride, glycerin, and a non-aqueous solvent. Preferably the aqueous solution contains glycerin and sodium chloride. Preferably, a sufficient amount of propylene glycol is present in the aqueous solution, prior to addition of the solution from step 1, in order to achieve the final desired concentration of propylene glycol.

The order of addition of desired components is not expected to seriously impact the resulting lipid suspension. However, it is preferred that the lipid-blend solution is added to water, which may already contain the above-noted additional components. Additional desired components can then be added. It is more preferred that the lipid-blend solution is added a solution of water and sodium chloride (i.e., saline). It is further preferred that the lipid-blend solution is added a solution of water, sodium chloride, and glycerin. It is still further preferred that the lipid-blend solution is added a solution of water, sodium chloride, glycerin, and propylene glycol.

It is preferred that 6.8 mg of NaCl are present per mL of formulation. Preferably, 0.1 mL of Glycerin per mL of formulation is present. A final concentration of 0.1 mL of Propylene Glycol per mL of formulation is preferred. The final pH of the formulation is preferably about 5.5-7.0. The lipid blend is preferably present in an amount of 0.75-1.0 mg/mL of formulation.

The temperature of the aqueous solution can range from ambient to 70°C. Preferably, the temperature is about 45 to 60°C, with 50, 51, 52, 53, 54, or 55 being even more preferred. In order to obtain complete dissolution, the mixture will need to be agitated, preferably stirred. Also, the pH of the solution may need to be adjusted, depending on the desired final formulation. Either acid (e.g., HCl) or base (e.g., NaOH) can be added to make such an adjustment.

The lipid suspension will contain liquid particles of varying sizes. One of the benefits of the present invention is the ability to consistently obtain small particles of a nearly uniform size. Thus, it is preferred that the majority of particles obtained are less than 100 nm in diameter, more preferable less than 50 nm.

A preferred procedure for dissolving the lipid blend is as follows: (a) Add Water for Injection (WFI) into a compounding vessel. (b) Start mixing and ensure temperature is from 50-55°C. (c) Add sodium chloride to the compounding vessel. Wait until the solid has completely dissolved before proceeding to the next step. (d) Add glycerin to the compounding vessel. Allow sufficient time for complete mixing. (e) Add the remaining Propylene Glycol that is not in the Lipid Blend/Propylene Glycol solution. Allow time for thorough mixing. (f) Reduce mixing rate to reduce turbulence in the compounding vessel. (g) Add the Lipid Blend/Propylene Glycol solution to the compounding vessel. (h) Readjust mixing to original rate. (i) Add additional WFI if necessary. (j) Continue to mix for approximately 25 minutes and assure complete mixing. (k) Verify and adjust the solution to target pH.

Step(3):

Step three involves heating the lipid suspension obtained from step (2) to a temperature about equal to or above the highest gel to liquid crystalline phase transition temperature of the lipids present in the solution.

One of the objects of this step is to provide a filterable suspension. A solution/suspension is considered

filterable if there is no significant reduction in flow rate within a normal process, and there is no significant increase in the pressure drop in the filtration system.

Experimental data indicates that the lipids in the formulation should be beyond their gel to liquid crystalline phase transition in order to simplify sterile filtration. When the lipids are below the phase transition temperature, the suspension particles are rigid. However, when they are above their respective gel-liquid crystal phase transition temperatures, they are in a more loosely organized configuration and thus, more easily filtered.

DPPC and DPPA show phase transitions of 41°C and 67°C respectively. MPEG5000-DPPE is soluble in water, therefore it does not exhibit a gel-liquid crystal phase transition which is characteristic of most hydrated lipid suspensions. Because the lipids in the preferred formulation all exhibit different gel to liquid phase transitions, the highest phase transition temperature, 67°C, is preferably used to filter the solution. By maintaining temperature at or beyond 67°C, all the lipids are beyond their respective phase transition, assuring the loose configuration while passing through the filters.

Heating may be achieved by jacketing the compounding vessel with a heat exchanging coil. Hot water/steam from a controlled source, e.g., a hot water bath, or a water heater, would deliver sufficient heat to maintain the compounding solution at a set temperature. Other heat sources known to thsoe of skill in the art could also be used.

Step (4):

Step four is performed by filtering the lipid suspension through a sterilizing filter. The purpose behind this step being to provide a substantially bacteria-free suspension. A filtrate is considered substantially bacteria-free when the probability of the filtrate to contain at least one colony forming microorganism is less than 10^{-6}.

Filtration is preferably done using sterilizing filter cartridges. Also, a means of forcing the solution through the filters may be required (e.g., pumping or pressurizing). Since the solution being filtered needs to be maintained at a temperature at or above the highest gel to liquid crystalline phase transition temperature of the lipids present in the solution, the filtration should be performed at about this same temperature. In order to accomplish this, the filter (e.g., sterilizing filter cartridges) are preferably enclosed in jacketed filter housings which are continuously heated, e.g., by a hot water stream from a temperature controlled water bath, to ensure that the suspension is above the lipid phase transition temperatures. The temperature of the sterilizing filter is preferably from 50 to 100°C, more preferably from 60 to 90°C, and even more preferably 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, or 80°C.

One or more sterilizing filters may be used to filter the suspension. The required number will be based on their effectiveness at removing bacteria. It is preferred that two filters are used. The size of the filter pores will be limited by the need to provide a bacteria-free suspension. Preferably, 0.2µm hydrophilic filters are used.

A bulk solution of the preferred formulation was continuously filtered through two 0.2µm hydrophilic filters for up to 3 hours at a rate of approximately 1 liter per minute (1 L/min.), i.e., passing a total of 180 liters of the suspension solution through the filters. The experimental results shows that there is no apparent blockage of filters. Lipid assays indicates that there is no measurable loss during the filtration process (due to accumulation in the filter medium).

A bulk solution of the preferred formulation was compounded at 40°C-80°C, and the suspension was cooled to ambient temperature prior to sterile filtration. No apparent clogging of the filters were observed indicating the suspension particle size distribution is well below 0.2µm of the filter pore size. It is desirable to use heat during filtration in order to ensure maximum recover of the

lipid blend in the sterile filtrate (i.e., to minimize potential retention of lipid particles in the filter medium).

A preferred procedure for filtering the lipid suspension is as follows: (a) Assure all jacketed filters are at 70°C - 80°C. (b) Assure all valves in the filtration unit are closed. (c) Connect filtration inlet hose to the outlet of the compounding vessel. (d) Open valves to allow solution to pass through the filters. (e) Flush three liters of solution through the filters before collecting filtrate. (f) Continue filtration until complete.

Step (5):

Dispensing the filtered solution into a vial completes step five. Preferably, this step is performed in a controlled aseptic area. One of ordinary skill in the art would recognize that the vial selected and amount of suspension delivered to the vial would depend on the end use considered for the lipid suspension. Dispensing can be achieved via a variety of methods, including pipette, hand-held syringe dispenser (e.g., Filamatic® syringe dispensing machine), or industrial auto dispensing machine (e.g., Cozzoli or TL auto filling machine).

Step (6):

Step six is performed by exchanging the headspace gas of the vials from step five with a perfluorocarbon gas. A preferred method of exchange is to load the dispensed vials into a lyophilizer chamber and replace the vial headspace gas with a perfluorocarbon gas. A preferred gas is perfluoropropane (PFP). Other methods of headspace gas exchange known to those of skill in the art may be employed.

The vials are sealed at the completion of the vial headspace gas exchange cycle. When the lyophilizer chamber pressure is brought back to atmospheric pressure by charging into the chamber with PFP. Vial stoppers are seated to seal the vials.

Step (7):

Step seven involves terminally sterilizing a vial after step six. One method of terminal sterilization is through the use of an autoclave. Also, the sealed vials can be terminally sterilized in a steam sterilizer to further enhance the sterility assurance of the product. Care must be taken in the sterilization process as some degradation of lipids may be observed as a result of autoclaving. Preferably, the vial is sterilized at about 126-130°C for 1 to 10 minutes.

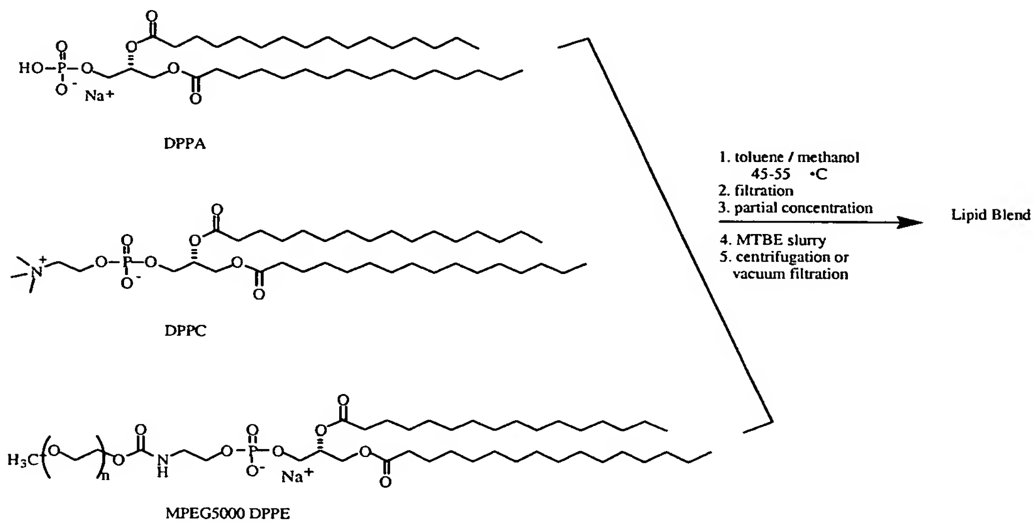
Other features of the invention will become apparent in the course of the following descriptions of exemplary embodiments which are given for illustration of the invention and are not intended to be limiting thereof.

EXAMPLES

Table 1: Lipid Blend Target Composition

Lipid Name	**Common Name**	**Wt %**	**Mole %**
DPPA	1,2-dipalmitoyl-*sn*-glycero-3-phosphatidic acid, monosodium salt	6.0	10
DPPC	1,2-dipalmitoyl-*sn*-glycero-3-phosphatidylcholine	53.5	82
MPEG5000 DPPE	*N* -(methoxypolyethylene glycol 5000 carbamoyl)-1,2-dipalmitoyl-*sn*-glycero-3-phosphatidylethanolamine, monosodium salt	40.5	8

Lipid Blend Manufacturing Procedure

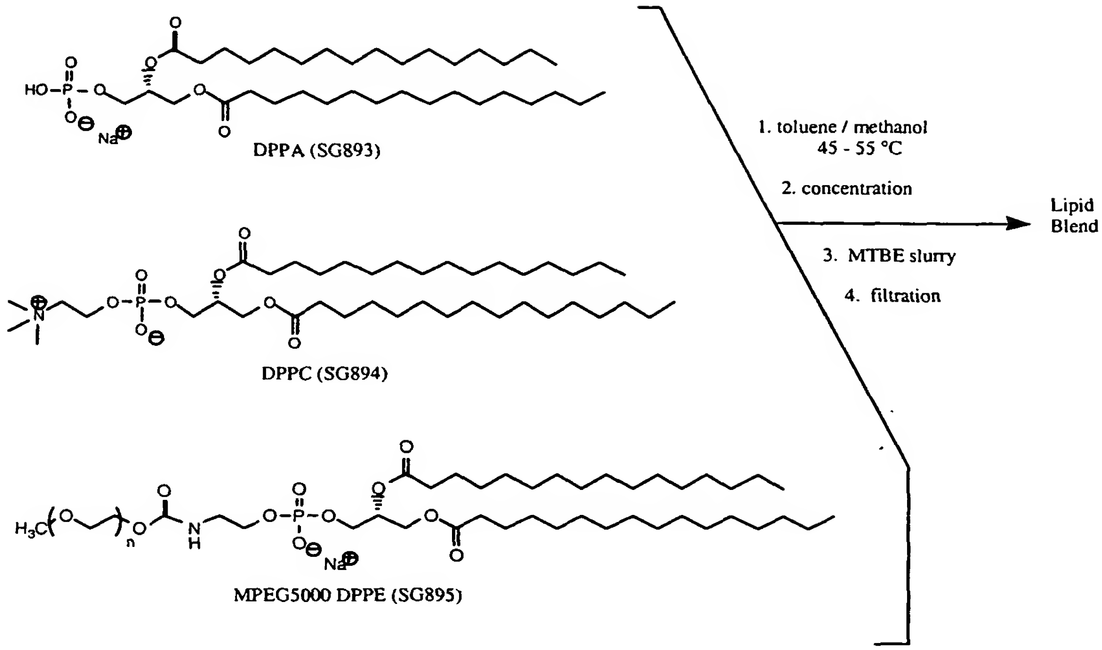


A flask is charged with toluene (3.3 L), methanol (1.2 L), DPPA (59.6 g), DPPC (535 g), and MPEG5000 DPPE (405 g). After rinsing solid contact surfaces with 0.9 L methanol, the slurry is warmed to 45-55°C until dissolution is complete.

The solution is filtered and then concentrated *in vacuo* at 35 - 45° C to a thick gel. Methyl *t*-butyl ether (MTBE, 5.4 L) is added and the mixture is slurried at 15-30°C. White solids are collected by centrifugation or vacuum filtration, and washed with MTBE (0.9 L). The solids are then placed in a vacuum oven and dried to constant weight at 40-50°C. The dried Lipid Blend is transferred to a bottle and stored at -15 to -25 °C.

In another embodiment of the lipid blend manufacturing procedure of the present invention, the following procedure may also be used.

Alternative Lipid Blend Manufacturing Procedure



Phospholipid quantities were adjusted for purity based on a "Use As" value from the certificates of analysis. The batch size (combined phospholipid weight) of this experiment was 2 kg.

A rotary evaporation flask is charged sequentially with toluene (3,300 mL), methanol (1,200 mL), DPPA (122.9 g; corrected for "use as" purity of 97.0 %), DPPC (1,098.5 g total; 500.8 g from a lot with 98.4 % "use as" purity and 597.7 g from a lot with 96.7 % "use as" purity), and MPEG5000 DPPE (815.7 g; corrected for "use as" purity of 99.3 %). After rinsing residual solids into the flask with methanol (900 mL), the flask is placed on a rotary evaporator (no vacuum) and the slurry is warmed to between 45 and 55 °C (external). After dissolution is complete, the external temperature is reduced to between 35 and 45 °C, a vacuum is applied, and the solution is concentrated to a white semi-solid. The flask is removed from the evaporator and solids are broken up with a spatula. The flask is reapplied to the evaporator and concentration is continued. After reaching the endpoint (final vacuum pressure = 20 mbar; white, granular, chunky solid), MTBE (5,400 mL) is

added through the rotary evaporator's addition tube, the vacuum is discontinued, and the mixture is slurried for 15 to 45 min at 15 to 30 °C. Solids are isolated by either centrifugal or vacuum filtration, rinsed with MTBE (3,800 mL), and dried to constant weight in a vacuum oven (40 to 50 °C). Prior to transferring to polyethylene bottles with polypropylene caps, solids are delumped through a screen (0.079 inch mesh), affording 1,966.7 g (98 %) of lipid blend (SG896) as a white solid.

The preferred lipid suspension contains:

1,2-dipalmitoyl-*sn*-glycero-3-phosphotidic, mono sodium salt (DPPA);

1,2-dipalmitoyl-*sn*-glycero-3-phosphatidylcholine (DPPC);

N-(methoxypolyethylene glycol 5000 carbamoyl)-1,2-dipalmitoyl-*sn*-glycero-3-phosphatidylethanolamine, monosodium salt (MPEG5000-DPPE);

Propylene Glycol, USP;

Glycerin, USP;

Sodium Chloride, USP; and,

Water for Injection, USP.

Table 2: Preferred Contrast Agent Formulations

Component	A*	B*
NaCl, USP	6.8 mg/mL	6.8 mg/mL
Glycerin, USP	0.1 mL/mL	0.1 mL/mL
Propylene Glycol, USP	0.1 mL/mL	0.1 mL/mL
Lipid Blend**	1 mg/mL	0.75 mg/mL
Perfluoropropane	> 65%	> 65%
pH	6.0 - 7.0	6.0 - 7.0

*Formulation A has 1 mg/mL lipid blend. Formulation B has a lipid blend concentration of 0.75 mg/mL.

**The lipid blend is consist of 53.5 wt.% of DPPC, 6.0 wt.% of DPPA and 40.5 wt.% of MPEG5000-DPPE.

Table 3: Preferred Container and Closure

Component	Type
Vial	Wheaton 2802, B33BA, 2cc, 13mm, Type I, flint tubing vial
Stopper	West V50 4416/50, 13mm, gray butyl lyo, siliconized stoppers
Seal	West 3766, white 13mm, flip-off aluminum seals

The finished product fill volume can be from 1.0-2.0 mL/vial.

In the preparation of the preferred formulation, when the lipid blend is directly hydrated with the aqueous matrix solution containing water for injection, sodium chloride, glycerin and propylene glycol, the filtrates have less lipids as compared to the pre-filtration bulk solution. The loss of lipids varies from 12% to 48%. These results demonstrate that the sterile filtration process is not effectively controlled, and therefore, the final product lipid content is highly variable.

In contrast, using the presently described process, assay results of the lipids in show full recovery of lipids during the filtration process. Variability of assay results around the theoretical targets is within normal assay method variability. Particle size distribution by number, by volume and by reflective intensity of a suspension prepared by first solubilizing lipid blend in propylene glycol indicate that the majority of the particles are less than 50 nm in the pre-filtered bulk solution at 55°C as well at 70°C. The particle distribution profile does not change after filtration.

UTILITY SECTION

The presently claimed process is useful for preparing ultrasound contrast agents. Such agents should be useful

for a variety of imaging applications, including enhancing contrast in echocardiographic and radiologic ultrasound images.

Obviously, numerous modifications and variations of the present invention are possible in light of the above teachings. It is therefore to be understood that within the scope of the appended claims, the invention may be practiced otherwise that as specifically described herein.